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Isolation, identification and bioactivity of fungal endophytes from selected Kenyan medicinal plants

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Infectious diseases remain a global health burden due to development of antibiotic resistance by pathogenic microorganisms. Antibiotic resistance has led to increased number of deaths among children and adults. This study sought to isolate and identify fungal endophytes from selected Kenyan medicinal plants and screen them and their extracts for bioactivity against selected test human pathogens. Fifty-five fungal endophytes were successfully isolated from fresh leaves of twenty-three medicinal plants from Kakamega forest. The DNA of fungal endophytes was extracted and molecular characterization was done through sequencing of the internal transcribed spacer region (ITS). The isolated fungal endophytes belonged to nine genera in the Ascomycota group, namely *Fusarium*, *Colletotrichum*, *Trichothecium, Phomopsis*, *Pestalotiopsis*, *Cladosporium*, *Aspergillus*, *Phoma,* and *Chaetomium*. Extracts from *Aspergillus* sp. demonstrated antimicrobial activity at low concentrations of 2.34 µg/ml against *B. subtilis* and 9.38 µg/ml against *Candida tenius*; while extracts from *Colletotrichum* sp. demonstrated antimicrobial activity at moderate concentration (37.5 µg/ml) against *B. subtilis* in the serial dilution assay. These results show that medicinal plants are a reservoir to a diversity of fungal endophytes that could be exploited as sources of natural products of pharmaceutical importance.

Key words: Antibiotic resistance, antimicrobial, bioactive, fungal endophytes.

INTRODUCTION

Infectious diseases cause about 18.4% deaths worldwide (WHO, 2014) and are linked to the development of multidrug-resistant pathogenic bacteria. Several research initiatives have shown results of drug resistance in human pathogenic bacteria around the world (Laxminarayan et al., 2013). Consequently, attention has been focused on finding alternative antimicrobial compounds from natural sources including endophytes. Fungal endophytes are a group of microorganisms that live in tissues of healthy plants, inter and intra-cellularly

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without causing any disease symptom (Wilson, 1995). Additionally, it is postulated that medicinal plants and endophytes display a mutualistic relationship. In this case, plants act as hosts to these endophytes by providing nutrients and in return, the endophytes produce secondary metabolites, which protect the plants against pathogenic herbivore attack (Yu et al., 2010). Therefore, there is a possibility of fungal endophytes isolated from medicinal plants to produce natural products of pharmaceutical significance.

Medicinal plants growing in natural habitats such as the Kakamega Tropical Rainforest are promising hosts of fungal endophytes that might produce bioactive secondary metabolites of pharmaceutical importance. Additionally, the local community in the treatment of various illnesses uses these plants. Therefore, micro-organisms chosen in this study are a representation of the common pathogens that cause infectious diseases. In this paper, the isolation, identification, and antimicrobial screening of fungal endophytes from Kenyan medicinal plants was reported.

MATERIALS AND METHODS

Isolation of the fungal endophytes

Twenty-three medicinal plants were selected for this study based on available ethno botanical information. Furthermore, fresh and healthy leaves were selected with the help of a plant pathologist. Sampling was done at Kakamega Tropical Rainforest, which lies at 0°10' to 0°21'N 34°58' E. Isolation of fungal endophytes was performed by a modification of the method described by Zinniel et al. (2002). In this method, the leaves of the selected plants were washed under running tap water to remove any soil or other foreign materials. Afterwards, they were surface sterilized for three minutes using 70% ethanol followed by soaking in 1% sodium hypochlorite for one minute and rinsed three times with sterile distilled water to remove any traces of the disinfectant. The surface sterilized leaves were then cut aseptically into sections approximately 1 mm by 4 mm and plated in Petri dishes containing potato dextrose agar (PDA) amended with streptomycin sulphate (250 mg/l). The inoculated plates were placed in an incubator at 25 ± 2°C for 1 to 4 weeks. The Petri dishes were monitored after every three days to check for fungal growth. Pure cultures were then prepared by sub-culturing fungal mycelia of each endophyte isolate into sterile Petri dishes containing PDA without antibiotics.

Identification of the fungal endophytes

Identification of the fungal endophytes was done using molecular characterization through sequencing of the internal transcribed spacer region (ITS). However, morphological identification was not possible in this study because the endophytes did not produce fruiting structures in culture.

DNA extraction and amplification

DNA extraction of endophytic fungi was done using BIO BASIC EZ-10 Spin column miniprep kit following the manufacturer's instructions (Bio Basic Inc.). Fungal endophyte mycelia were obtained from one-week-old cultures and 60 mg of my celium were used for DNA extraction. The internal transcribed spacer (ITS) region of the ribosomal RNA operon was amplified using two primers, ITS1F (CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Polymerase Chain Reaction (PCR) was carried out in a thermal cycler (Applied Biosystems^{TM}) as follows: An initial denaturation of 5 min at 94°C, followed by 35 cycles of denaturation for 3 s at 94°C, 1 min for annealing at 52°C, 1 min for elongation at 72°C and a final elongation of 10 min at 72°C. The quality and quantity of PCR products (3 µL) were checked by electrophoresis on a 0.8% agarose gel stained with Midori green dye and visualization was done with an UV transilluminator (Nippon Genetics Europe GMbH).

Sequencing and phylogenetic analysis

The amplified PCR products were purified according to BioBasic EZ-10 spin column PCR purification kit following manufacturer's instructions. Sequencing was done using the automated illumina genome analyzer IIX DNA sequencing machine (hiseq). Sequences were compared to ITS sequence data from strains available in the public databases GenBank (http://www.ncbi.nlm.nih.gov/genbank/) by using Basic Local Alignment Search Tool (BLAST) N sequence match routines. The sequences were then aligned using ClustalW software program. Alignments obtained were used to calculate distance matrices and construct phylogenetic trees. Phylogenetic and molecular evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura et al., 2013).

Fermentation and extraction of fungal endophytes

Submerged fermentation was carried out using the method described by Stadler et al. (2001) followed by extraction in acetone and ethyl acetate. The inocula were prepared by introducing 7 mm mycelial agar plugs from 10-day-old fungal culture into 250 mL Erlenmeyer flasks containing 200 mL sterilized sugar malt (SM1/2) and yeast malt (YM6.3) broth medium. The cultures were cultivated at 23°C with a rotational speed of 140 rpm. After 10 to 14 days of incubation, the fungal biomass and the fermentation broth were separated by filtration. The fungal biomass was extracted by soaking in 200 mL acetone followed by mixing on a magnetic stirrer for 5 min and then subjected to an ultrasonic sound bath for 30 min. The fungal biomass was separated from acetone by filtration, then the filtrate which constituted acetone was concentrated by evaporation on a vacuum rotavapor at 40°C and pressure of 540 pa leaving behind the water phase. An equal volume of ethyl acetate was added to the water phase, and the upper organic phase was then concentrated to dryness. The dry extract obtained was weighed and stored at -20°C for further analysis. The final pH of the fermentation broth was measured, followed by extraction with equal volume of ethyl acetate. The upper organic phase was concentrated to dryness on a vacuum rotavapor at 40°C and a pressure of 240 pa. The dry extract obtained was weighed and stored at -20°C for further analysis.

Screening crude extracts of fungal endophytes for antimicrobial activity and minimum inhibitory concentration (MIC) test

Antimicrobial activity of fungal endophyte extracts was determined using the serial dilution assay test as described by Okanya et al. (2011). The test pathogens used were *Bacillus. subtilis* DSM10, *Escherichia. coli* DSM498, *Candida. tenius* MUCL29892, and *Mucor.*

Table 1. Fungal endophyte isolation frequency and their plant sources.

Plant source	No. of endophytes isolated
Piper capense (Wild pepper)	4
Kigelia africana (Sausage tree)	2
Tragia insuavis	3
Teclea nobilis	3
Polyscias fulva (Parasol tree)	2
Afromonum angustifolium	1
Trichilia emetica (Natal mahogany)	4
Markhamia lutea (Nile Tulip)	3
Toddalia asiatica (Orange climber)	2
Clausena anisata	5
Albizia gummifera (Smooth barked flat-crown)	3
Revolva caffra	2
Vernonia amygadalina (Bitter leaf)	1
Tithonia diversifolia	2
Barsamia alba	1
Mondia wheitei (White's ginger)	3
Warburgia ugandensis (Uganda greenheart)	4
Prunus africana (Iron wood)	3
Croton macrostachyus (Broad leaved croton)	2
Zanthoxylum gilletii (African Satinwood)	2
Brugmansia sp.	2
Erythrococca sp.	1
Total	**55**

plumbeus MUCL4935. Overnight 24 h cultures of *B. subtilis* DSM10 and *E. coli* DSM498 were prepared by inoculating 1 ml of the stock cultures in sterile 100 ml EBS media. The cultures were then incubated at 30°C for 24 h. Fungal cultures of *C. tenius* MUCL29892 and *M. plumbeus* MUCL4935 were prepared by inoculating 1 ml of the stock cultures in sterile 100 ml of yeast malt media. Thereafter, the cultures were incubated at 23°C for 48 h. Bacterial cell suspensions were diluted to 10^5 CFU/ml and 280 µl of each cell suspension was pipetted into the first row (A1-A12) of a 96-well plate. In addition, 20 µl of 4.5 mg/ml crude fungal endophyte extracts was pipetted into the first row to make a final concentration of 300 µg/ml. Thereafter, a 2-fold serial dilution with concentrations ranging from 300 to 2.34 µg/ml was made. Cycloheximide and Nystatin were used as reference antibiotic and antifungal respectively with concentrations ranging from 100 to 0.78 µg/ml. The 96-well plates with bacterial pathogens were incubated at 30ºC for 24 h, whereas plates with fungal pathogens were incubated at 23°C for 48 h. The presence of clear wells was used as an indicator of antimicrobial activity. Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of fungal endophyte extract that inhibited visible growth.

Data analysis

Mean inhibition zones were calculated and equality of means was analyzed using one way analysis of variance (ANOVA).Tukey's Honestly Significant Difference (HSD), a post-hoc analysis, was used to determine if there was any significant difference between the means of the isolates. Data analysis was performed using R statistical software version 3.3.1.

RESULTS

Fifty-five fungal endophytes were successfully isolated from surface sterilized fresh leaves of 23 indigenous medicinal plants sampled in Kakamega Tropical Rainforest, Kenya (Table 1). *Warburgia ugandensis*, *Piper capense*, *Clausena anisata* and *Trichilia emetica* were among the medicinal plants that yielded the highest number of fungal endophyte isolates.

Successful PCR amplification and ITS sequences were obtained for 49 of the isolated fungal endophytes. The ITS PCR products after size separation by agarose gel electrophoresis showed that the PCR products gave the expected DNA band size (500-700 bp) indicating that the primers used successfully amplified the ITS region. The ITS1-5.8S-ITS2 sequences of the isolates were compared to ITS sequences of other organisms represented in the NCBI database GenBank. BLAST results revealed that isolated fungal endophytes belonged to three classes of Ascomycota; Eurotiomycetes, Sordariomycetes and Dothideomycetes. These included nine genera; *Colletotrichum* (44%), *Fusarium* (32%), *Phomopsis* (7%), *Pestalotiopsis* (7%), *Aspergillus* (2%), *Chaetomium* (2%), *Cladosporium* (2%), *Trichothecium* (2%) and *Phoma* (2%) (Figure 1).

Seventeen endophytic fungal isolates (28%) demonstrated antagonism against *S. aureus* ATCC25923

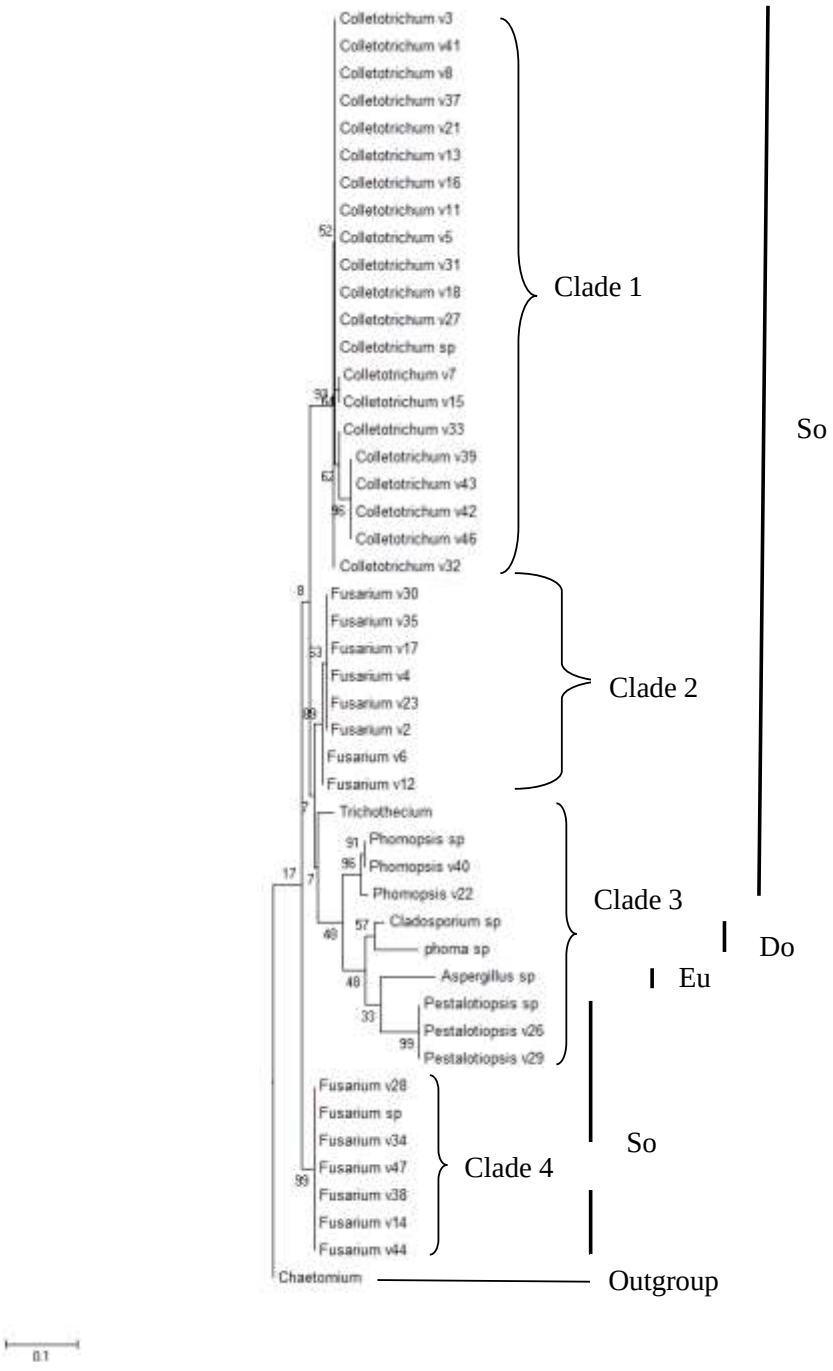

Figure 1. Phylogenetic tree of the isolated fungal endophytes based on ITS analyses (Maximum Likelihood method; 1000 replicate bootstrap). Class: So, Sordariomycetes; Eu, Eurotiomycetes; Do, Dothidiomycetes.

(Table 2). This activity was not significantly different (P< 0.01) from the positive control (chloramphenicol) with inhibition zone >14 mm. Endophytic fungal isolates of the genera *Pestalotiopsis*, *Colletotrichum*, and *Fusarium* were the most active; *Fusarium* sp. (ML1) exhibited a maximum inhibition zone of 17.7±1.20 mm. Besides, antagonism of twenty-two endophytic fungal isolates (36.7%) against *P. aeruginosa* ATCC87853 (Table 2) was not significantly different (P< 0.01) from the positive control. Maximum activity was registered by *Colletotrichum* sp. (PC5) with an inhibition diameter of 17.0±0.58 mm. Isolates of the genera *Phomopsis* sp., *Chaetomium* sp. and *Cladosporium* sp. showed minimal antibacterial activity with significant differences in their

Table 2. Inhibition diameters (mm) ± standard error (se) for the isolates extracts against the test organisms.

Test organism (diameter in mm, n=3)			
Isolate	***S. aureus***	***Isolate***	***P. aeruginosa***
Fusarium sp.(ML1)	17.7±1.20^{ab}	*Colletotrichum* sp. (PC5)	17.00±0.58^{ab}
Colletotrichum sp. (ZG2)	16.3±1.86^{bc}	*Colletotrichum* sp. (RC1)	17.00±1.15^{ab}
Fusarium sp. (MW2)	16.00±1.00^{cd}	*Fusarium* sp. (BS2)	16.67±0.33^{ab}
Colletotrichum sp. (PC4)	16.00±2.30^{cd}	*Fusarium* sp. (CM3)	16.33±0.67^{bc}
Fusarium sp. (BS2)	15.67±1.20^{de}	*Fusarium* sp. (TN3)	16.33±0.33^{bc}
Fusarium sp. (CM3)	15.67±1.67^{de}	*Colletotrichum* sp. (WU1)	16.33±0.88^{bc}
Colletotrichum sp. (PA5)	15.67±0.88^{de}	*Trichothecium* sp. (CM2)	15.67±0.88^{cd}
Colletotrichum sp. (PC3)	15.67±2.7^{de}	*Fusarium* sp. (PA1)	15.67±0.67^{cd}
Pestalotiopsis sp. (TD1)	15.67±0.88^{de}	*Fusarium* sp. (AA)	15.33±1.20^{de}
Fusarium sp. (MW4)	15.33±0.33^{de}	*Pestalotiopsis* sp. (BA2)	15.33±0.33^{de}
Fusarium sp. (PC1)	15.00±0.58^{ef}	*Fusarium* sp.(PC1)	15.33±0.67^{de}
Colletotrichum sp. (PC2)	14.67±3.28^{fg}	*Colletotrichum* sp. (TI1)	15.33±1.67^{de}
Colletotrichum sp. (PC5)	14.67±0.33^{fg}	*Fusarium* sp. (ML4)	14.67±0.33^{ef}
Pestalotiopsis sp. (TD2)	14.33±2.33^{fg}	*Colletotrichum* sp. (PC2)	14.67±0.67^{ef}
Colletotrichum sp. (KA1)	14.00±1.73^{gh}	*Colletotrichum* sp. (TA2)	14.67±0.88^{ef}
Colletotrichum sp. (TA1)	14.00±1.53^{gh}	*Fusarium* sp. (BS1)	14.33±0.67^{fg}
Fusarium sp. (TN1)	13.67±2.40^{gh}	*Colletotrichum* sp. (PA4)	14.33±0.33^{fg}
Chloramphenicol	22.5±0.07^{a}	*Colletotrichum* sp. (PA5)	14.33±0.88^{fg}
Negative control	0.00±0.00^{i}	*Colletotrichum* sp. (CA1)	14.00±3.21^{gh}
		Colletotrichum sp. (CA3)	14.00±1.00^{gh}
		Colletotrichum sp. (PA2)	14.00±1.00^{gh}
		Colletotrichum sp. (TN2)	14.00±1.00^{gh}
		Chloramphenicol	19.7±0.88^{a}
		Negative control	0.00±0.00^{i}

Within a column, fungal endophytes sharing the same letter(s) are not significantly different in their inhibition while those with different letters are significantly different (α =0.05, Tukey HSD test) ± Standard error (se).

means from the positive control. Some *Fusarium* sp. (BS1, MW1, and WU2) and *Colletotrichum s*p. (TE4) were inactive against the two bacteria.

Ethyl acetate extracts of fungal endophyte isolates including *Colletotrichum* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. showed antimicrobial activity against *B. subtilis* DSM10, *E. coli* DSM498, *C. tenius* MUCL29892, and *M. plumbeus* MUCL4935. Supernatant extracts of *Pestalotiopsis* sp. isolated from *Tithonia diversifolia* showed antibacterial activity against *B. subtilis* at high concentrations with MIC values of 75 and 150 µg/ml. The extract was not active against *E. coli*, *C. tenius*, and *M. plumbeus*.

Extracts of *Aspergillus* sp. isolated from *Albizia gummifera* was the most effective against all the four test microorganisms. The supernatant extract was active against *B. subtilis* at low concentrations with MIC value of 9.38 µg/ml in YM6.3 media and a value of 2.34 µg/ml in SM1/2 media. Similarly, mycelial extract of the same isolate against *C. tenius* recorded MIC value of 9.38 µg/ml. In contrast, the antibacterial activity of the same extracts against *E. coli* was demonstrated at high concentrations of 75 µg/ml (Table 3). The same case was observed with antifungal activity at high concentrations of150 µg/ml against *M. plumbeus*.

Supernatant extracts in SM1/2 media of *Colletotrichum* sp. isolated from *Kigelia africana* demonstrated antibacterial activity against *B. subtilis* at a high concentration of 300 µg/ml. In contrast, supernatant extracts from YM6.3 media were active against *B. subtilis* at much lower concentration, recording MIC value of 37.5 µg/ml. However, extracts of these isolates were not active against *E. coli*, *C. tenius*, and *M. plumbeus*. Extracts of *Phomopsis* sp. isolated from *Trichilia emetica* were active against both *B. subtilis* and *M. plumbeus* at high concentrations of 75 and 150 µg/ml (YM6.3 media) and 150 µg/ml (SM1/2 media) respectively.

DISCUSSION

Currently, molecular identification techniques stand out as the most effective method for identification of non-sporulating fungal endophytes and the detection of viable but non-culturable fungi (Grunig et al., 2002). Therefore, fungal endophytes in this study were subjected to

Table 3. Minimum inhibition concentration (MIC) values of fungal endophyte extracts against selected test bacteria and fungi.

				MIC values (µg/mL)			
Isolate	**Endophyte**	**Media**	**E**	*B. subtilis*	*E. coli*	*M. plumbeus*	*C. tenius*
TD1	*Pestalotiopsis* sp.	YM 6.3	S	150	*	*	*
			M	*	*	*	*
		SM1/2	S	75	*	*	*
			M	*	*	*	*
KA1	*Colletotrichum* sp.	YM 6.3	S	37.5	*	*	*
			M	*	*	*	*
		SM1/2	S	300	*	*	*
			M	*	*	*	*
AG2	*Aspergillus* sp.	YM 6.3	S	9.38	75	*	150
			M	2.34	*	*	*
		SM1/2	S	2.34	18.75	150	75
			M	2.34	75	*	9.38
TE5	*Phomopsis* sp.	YM 6.3	S	75	*	150	*
			M	150	*	*	*
		SM1/2	S	150	*	*	*
			M	150	*	*	*
TE2	*Phomopsis* sp.	YM 6.3	S	*	*	*	*
			M	150	*	*	*
		SM1/2	S	*	*	*	*
			M	300	*	*	*
KA2	*Phomopsis* sp.	YM 6.3	S	150	*	*	150
			M	*	*	*	*
		SM1/2	S	*	*	*	300
			M	*	*	*	*

E, Extracts; S, Supernatant; M, Mycelia; *, No activity; SM, Sugar malt media; YM, Yeast malt media.

molecular identification based on sequencing the ITS1-5.8S-ITS2 region. The latter generates a considerable sequence variation between closely related species due to a faster rate of evolution thus, sequences of these regions provide a good resolution at lower taxonomic levels (genus and species level). Use of ITS sequences, however, have shortcomings in that they might not achieve a perfect sequence alignment at higher taxonomic levels such as family, order and class (Lindahl et al., 2013) due to high ITS variability. Also, about 20 to 30% of ITS sequences obtained from GenBank and other public databases for comparative analysis may not be accurate in their identification (Huang et al., 2009), a challenge that was equally encountered in this study as well. The drawback is attributed to the fact that most of the sequences deposited in these public databases fail to match the organism in question. Moreover, previous studies have indicated that the little ITS variation in ascomycete fungi makes the region undesirable for taxonomic identification at the species level (Jang et al., 2014). Similar to this study, isolated fungal endophytes were classified under the phylum Ascomycota whose identification was only achieved at the genus level.

In this study, DNA extraction and PCR amplification were successful; only about 4% of the fungal endophyte isolates were not amplified. Lack of PCR amplification could be due to some of the very likely reasons such as the bias of primers which may amplify ITS regions towards certain groups of fungi (Bellemain et al., 2010). Based on our results, ITS1F and ITS4 were amplified more efficiently in the three classes from the phylum Ascomycota. Some ITS primers like ITS1 and ITS5 are usually biased towards the amplification of Basidiomycetes while others, such as ITS2, ITS3, and ITS4 lean towards Ascomycetes (Bellemain et al., 2010). Another reason could be probably due to the production of PCR inhibitory secondary metabolites in culture (Paterson, 2004). Forty-nine PCR products were subjected to DNA sequencing, which yielded 47 readable sequences that were compared with other sequences in the NCBI database for identification. Two PCR products had no readable sequences; this could be due to errors

that might have occurred during the sequencing process considering that the process is sensitive. Another reason might be weak PCR products obtained during amplification.

BLAST analysis revealed high percentage similarity of different species among the same genera, suggesting that ITS region of some isolates is conserved. A phylogenetic tree was inferred based on the DNA sequences obtained. Fungal endophytes in the genera *Pestalotiopsis* (99%), *Phomopsis* (96%) and *Fusarium* (99%) exhibited high bootstrap values, whereas *Aspergillus* (33%) and *Chaetomium* (17%) showed low bootstrap values. Bootstrap values represent phylogenetic accuracy (Felsenstein, 1985); bootstrapping test whether the dataset supports the generated tree. Values above 50% are likely to indicate reliable groupings (Hillis and Bull, 1993) while lower values mean that the node in question was found in less than half of the bootstrap replicates. Concerning the findings obtained in the current study, it can be deduced that fungal endophytes belonging to *Fusarium*, *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Cladosporium*, and *Phoma* were reliably grouped (bootstrap values > 50%) as opposed to *Chaetomium* and *Aspergillus* (bootstrap values < 50%).

A majority of the isolates showed sequence homology to the genera *Colletotrichum* and *Fusarium* while a few sequences exhibited sequence homology with the genera Pestalotiopsis, *Phomopsis*, *Phoma*, *Cladosporium*, *Aspergillus*, and *Chaetomium*. Although a definitive taxonomic identification (at species level) of fungal endophytes isolated in this study was not achieved, the data generated indicate a great diversity of these organisms in the medicinal plants. These results, therefore, show that molecular markers are an essential part of the phylogenetic analysis, and it may lead to further research on endophytes, their characterization, and assessment of their genetic diversity. Further research should focus on definitive taxonomic identification methods using molecular markers such as β-tubulin gene and elongation factor 1α gene (EF-1α) in addition to the ITS rDNA gene in molecular identification of the isolates to the species level.

The fungal endophytes isolated in this study belong to commonly isolated taxa similar to *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Cladosporium*, *Chaetomium*, *Aspergillus* and *Fusarium*, which have been previously reported as endophytes in medicinal plants (Rakotoniriana et al., 2008). Most of the fungal endophytes such as those from the genera *Aspergillus*, *Colletotrichum*, *Pestalotiopsis*, and *Phomopsis* isolated in this study showed potential antimicrobial activity against test pathogens. Similarly, other studies have reported antimicrobial activity of extracts from these endophytes against pathogenic bacteria (Zhang et al., 2009; Chapla et al., 2014). Usually, in addition to plants producing their secondary metabolites, endophytes equally contribute to the production of secondary metabolites in plants. Secondary metabolite production by endophytes is primarily linked to reducing competition from other microorganisms within the host plants. Further, the production of these natural products defends both the host plant and endophytes from disease-causing microorganisms (Ludwig-Müller, 2015). The bioactivity displayed by extracts from the fungal endophytes in this study could contribute to the medicinal properties of the medicinal plants.

Extracts of *Aspergillus* sp. were the most effective against the test microorganisms with MIC values ranging between 9.38 µg and 2.34/ml. *Aspergillus* sp. has been reported to be major producers of mycotoxins harmful to humans. In the current study, *Aspergillus* sp. isolates exhibited the maximum antimicrobial activity against the test bacteria and fungi. There have been reports of antimicrobial activity of extracts from *Aspergillus* sp. For instance, compounds classified as tremorgenic mycotoxins were identified from the endophytic fungus *Aspergillus* sp. Some of these compounds were reported to have potent antibacterial activity against *E. coli* (Qiao et al., 2010). Other studies have documented bioactive secondary metabolites with structures similar to Ochratoxin A moiety, one of the most abundant mycotoxins found in food (Moore et al., 1972). Further, Traditionally, *A. gummifera*, *K. africana*, and *T. emetica* have been used in the treatment of ailments including stomachache, malaria, and dermatitis. Regarding these reports, this could mean that the bioactivity demonstrated by this fungal endophyte in this current study, could be due to the high production of mycotoxins as secondary metabolites.

Conclusion

From the results of this study, it can be concluded that medicinal plants are a host to a diverse group of fungal endophytes with varying degree of antimicrobial activity. An *Aspergillus* sp. isolates demonstrated a spiking antimicrobial effect against the test organisms. Besides, secondary metabolites produced by *Colletotrichum* sp., *Fusarium* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. isolates also showed antimicrobial activity against the test microorganisms. The bioactive endophytes should be identified to species level. Besides, the bioactive compounds produced by these endophytes should be further characterized for future study inference.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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